

PF研究会:放射光利用による構造生物学の将来像

# 放射光回折データから いかに最大の構造情報を引き出すか

—超高分解能および中・低分解能での結晶構造解析—

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2010.7.13 高エネルギー加速器研究機構研究本館小林ホール

# 含まれる情報を最大限に引き出す構造解析

## 高分解能・高精度構造解析

### 高精度の原子座標

高い信頼度の結合距離情報

原子振動・分子運動の評価

水素原子の実験的決定

電子密度解析(結合電子,  $d$  電子)

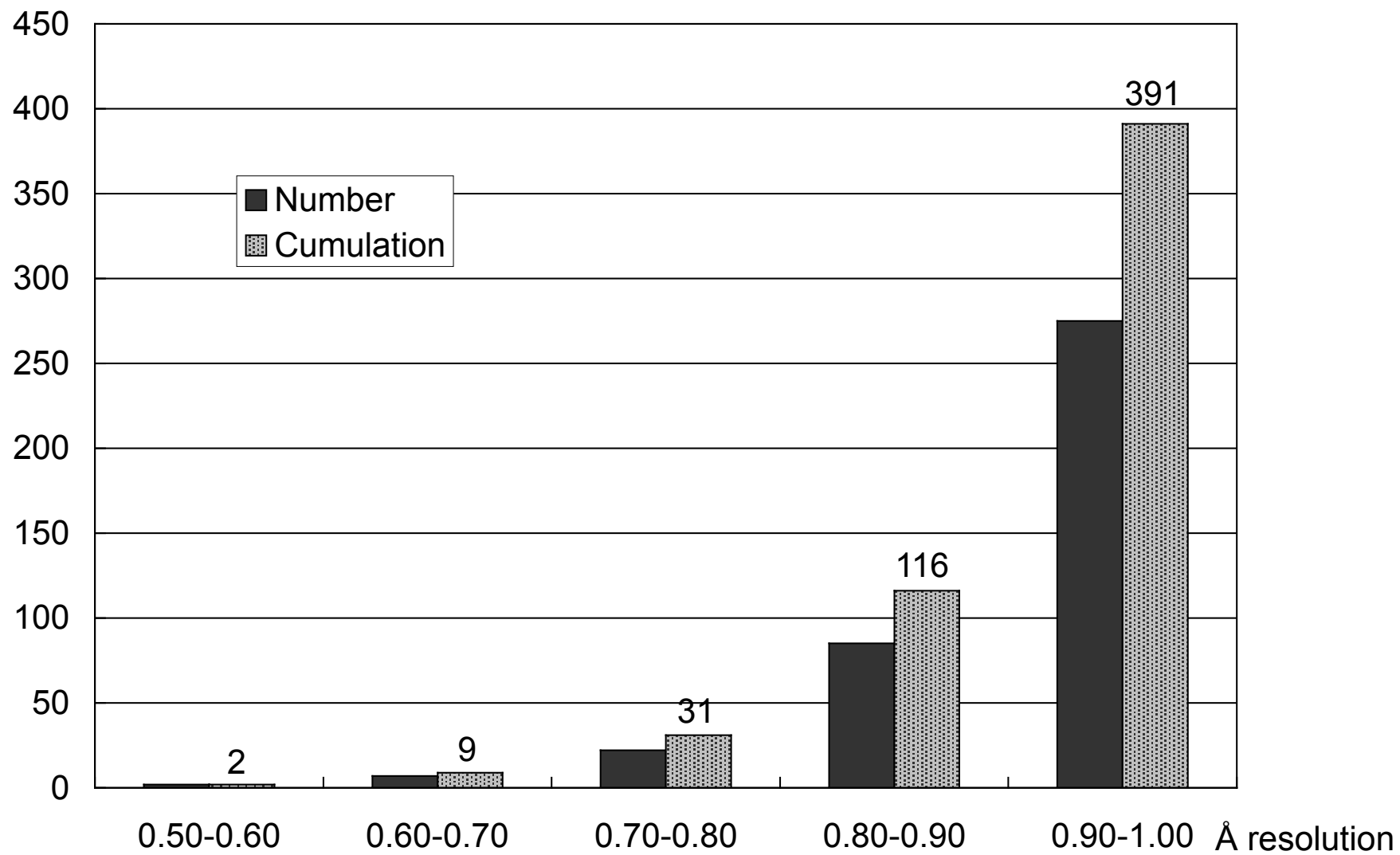
タンパク質分子での化学結合論

## 低分解能・中分解能構造解析

### あいまいな電子密度からの確かな構造情報

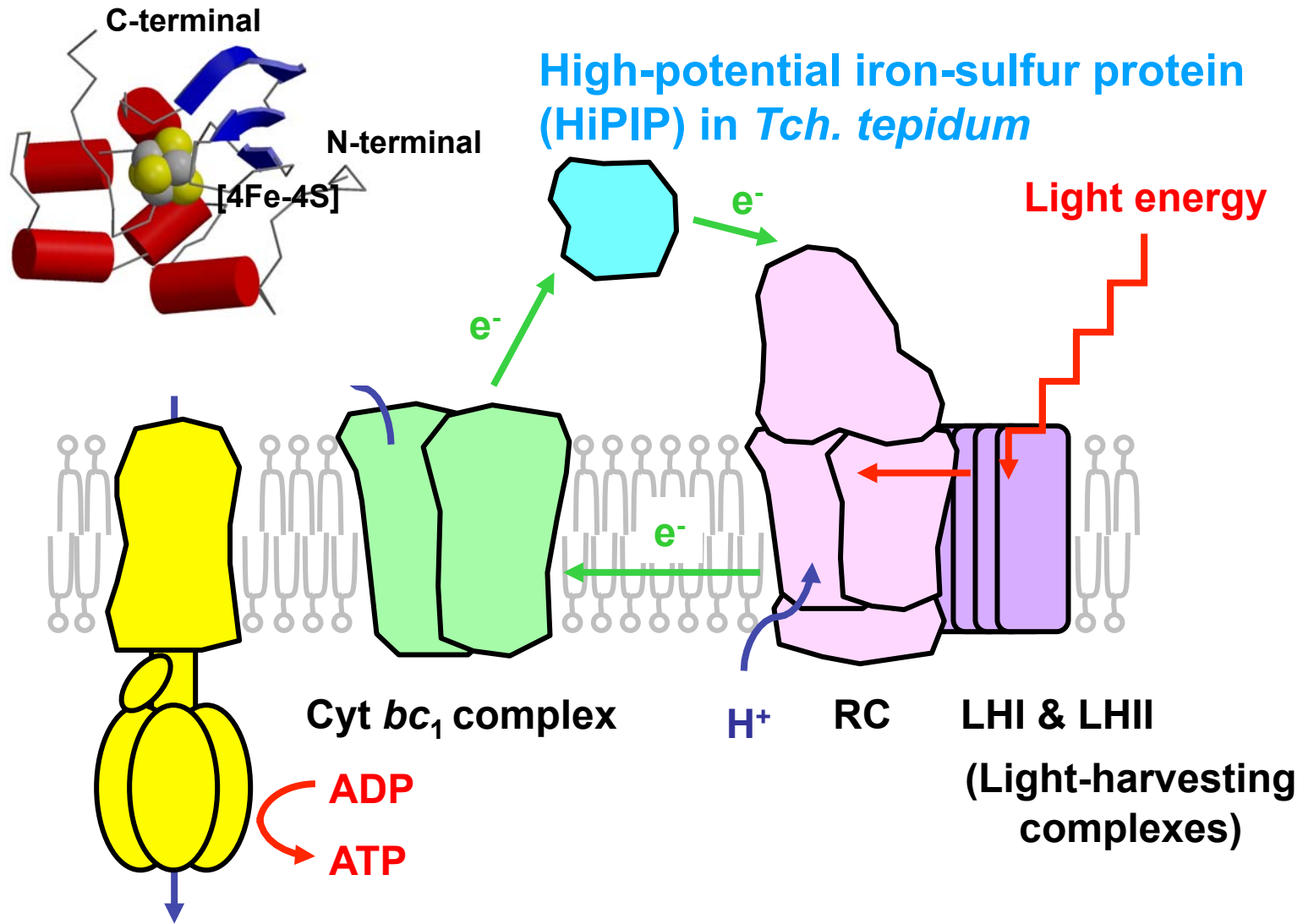
リガンド結合部位

二次構造, 三次(ドメイン)構造, 四次構造の変化



Structures  $\geq 1$  Å resolution are 0.69% of total entries for X-ray (56,680) in PDB on May 25, 2010

# Photosynthetic electron transfer in purple bacteria

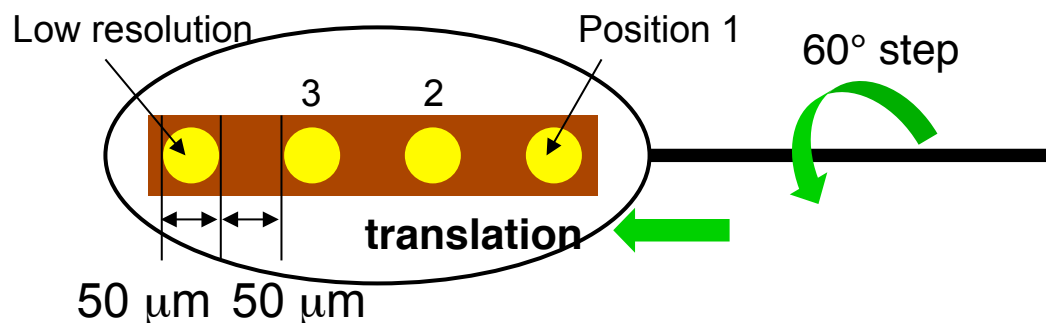


1.5Å分解能 (BL-6A, PF)  
0.8Å分解能 (BL40B2)

T.Nogi *et al.*, *Proc. Natl. Acad. Sci. USA*, 97,13561-13566 (2000).  
L. Liu *et al.*, *Acta Crystallogr.*, D58, 1085-1091 (2002).

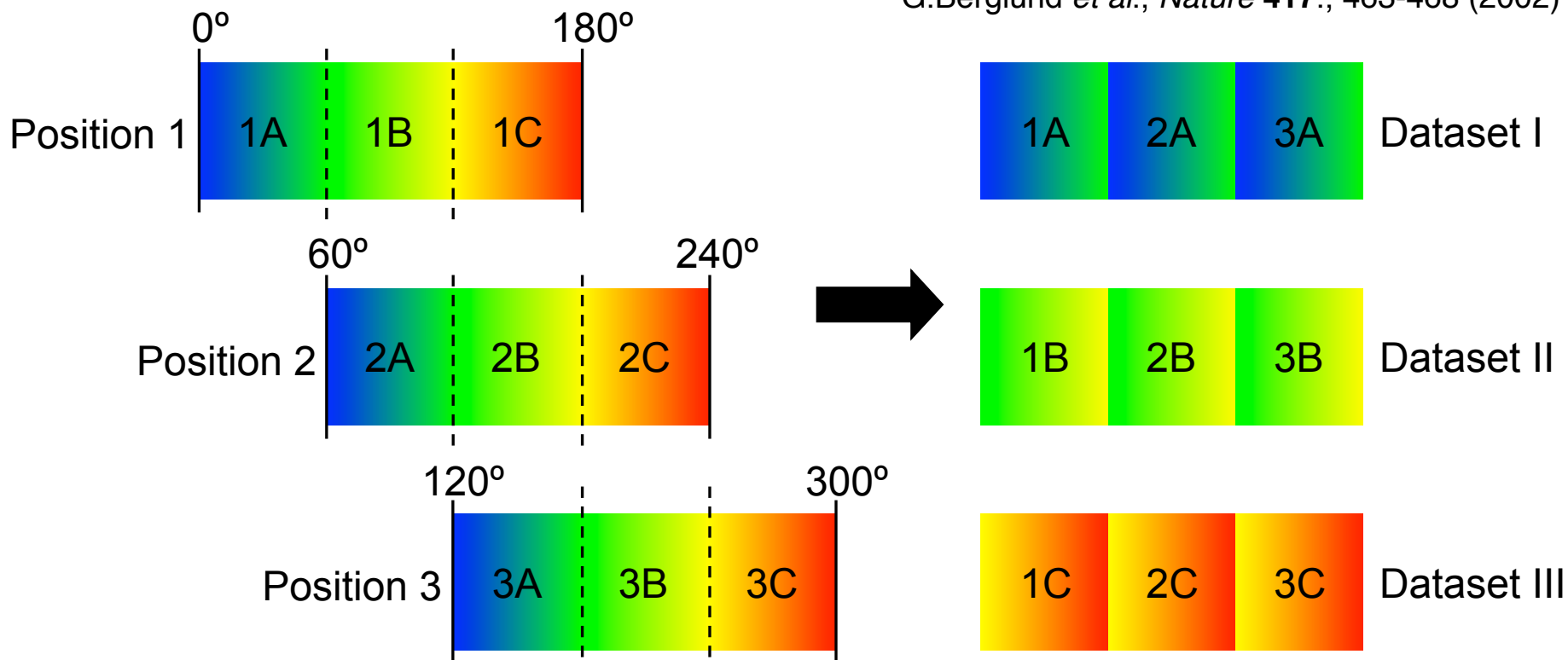
# HiPIP at 0.7 Å resolution:

Data collection to survey structural perturbation induced by X-ray damage

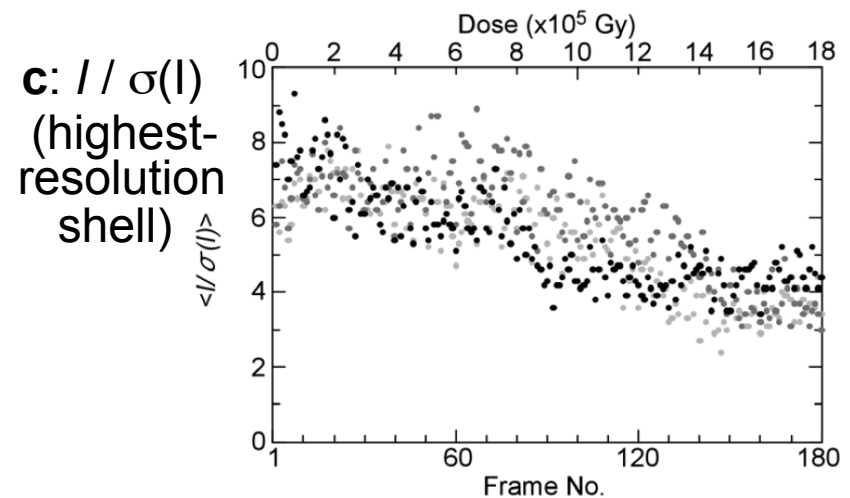
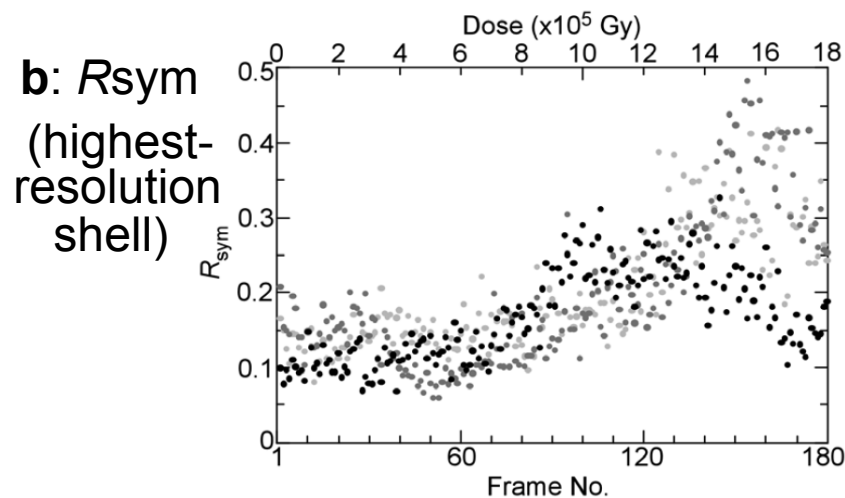
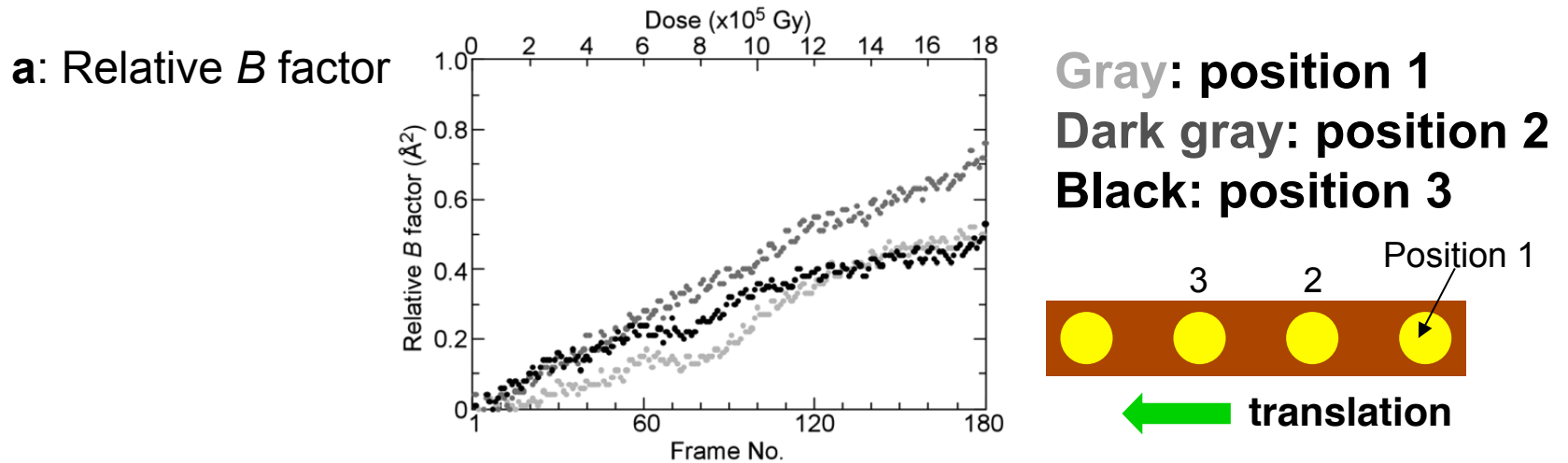


SPring-8 BL41XU  
Wavelength: 0.71 Å  
Detector: ADSC Q315  
Temperature: 93 K  
Oscillation range: 1°  
Exposure time: 2 sec

G.Berglund *et al.*, *Nature* **417.**, 463-468 (2002)



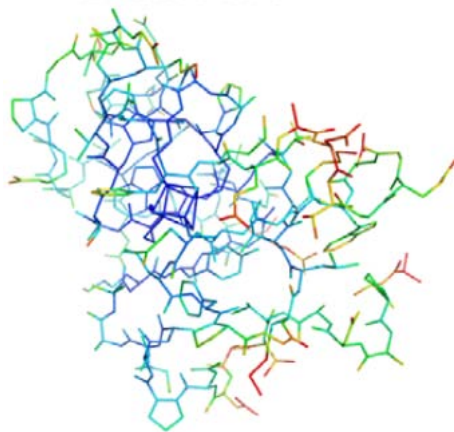
# HiPIP at 0.7 Å resolution: Changes in crystallographic parameters and statistics for the three incident positions of the crystal.



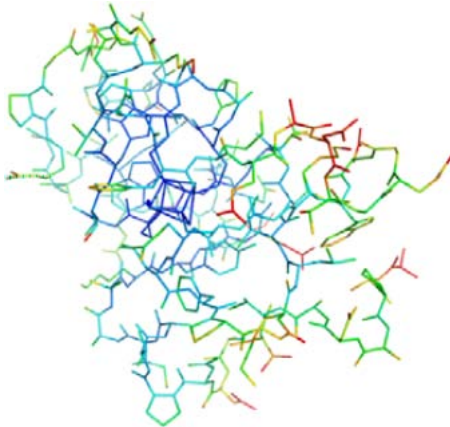
# HiPIP at 0.7 Å resolution:

Changes in temperature factors of protein molecule as exposure to X-rays

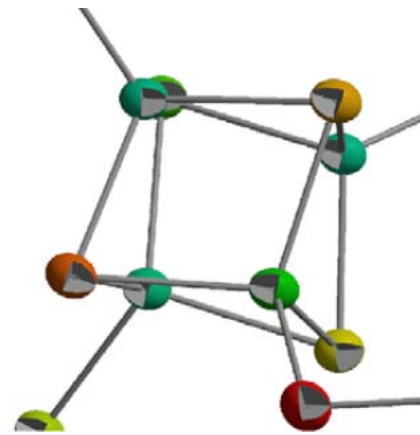
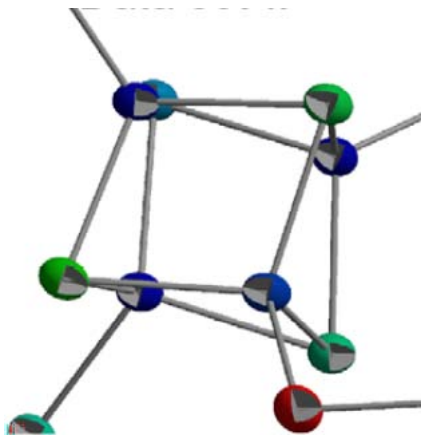
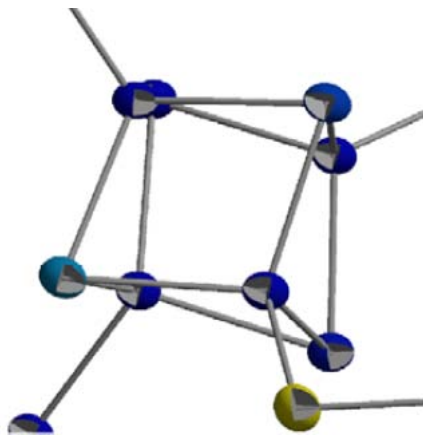
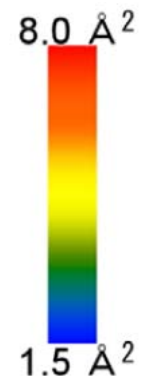
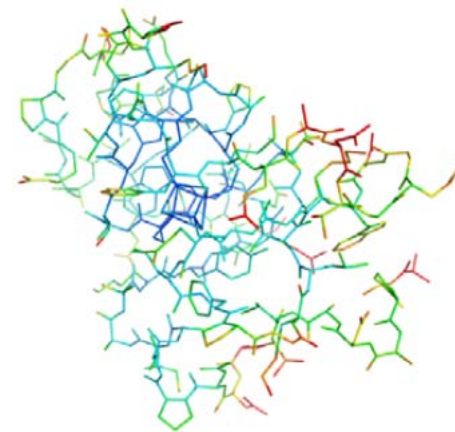
**Dataset I**



**Dataset II**

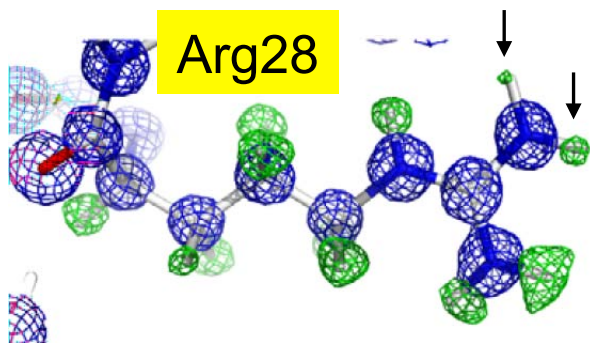


**Dataset III**

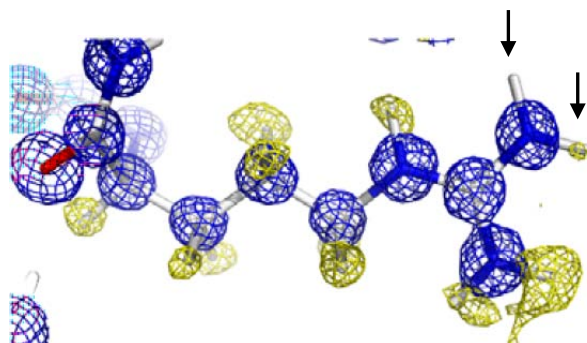


HiPIP at 0.7 Å resolution:  
The loss of electron density of hydrogen atoms  
around Arg28 and Trp74 by exposure to X-rays

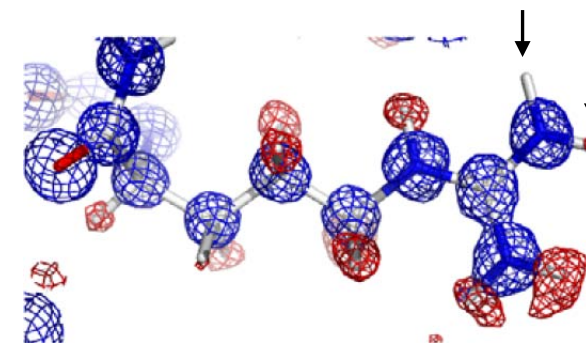
Dataset I



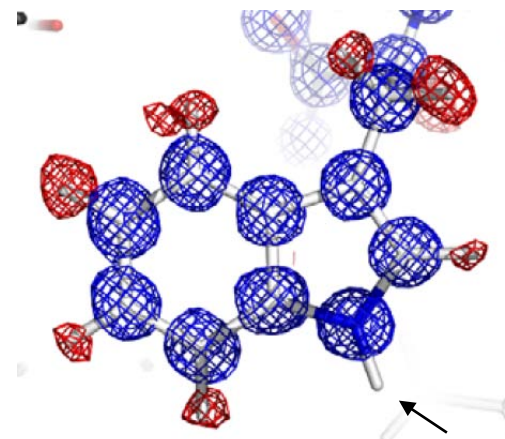
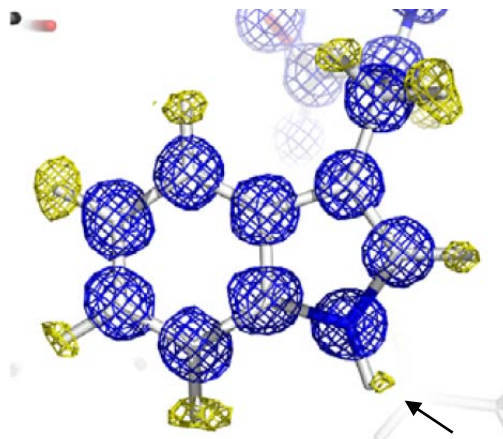
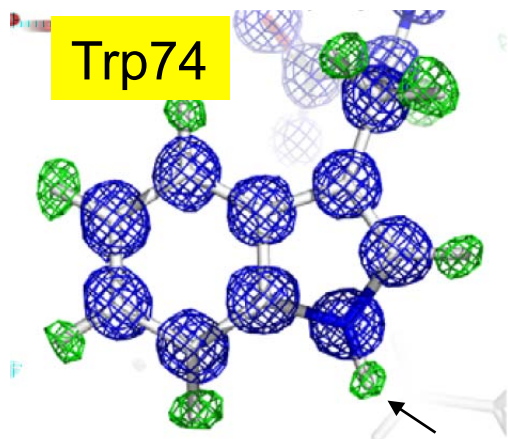
Dataset II



Dataset III



Trp74



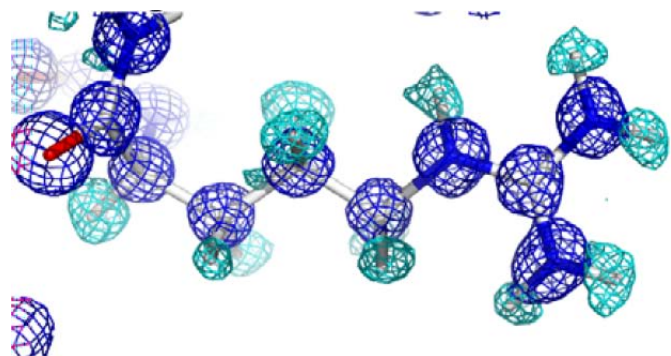
Blue: 2Fo-Fc contour 1.5  $\sigma$  ( $1.3 \text{ e}\text{\AA}^{-3}$ )

Green, Yellow, Red: Fo-Fc calculated by omitting hydrogen atoms contour 3.0  $\sigma$

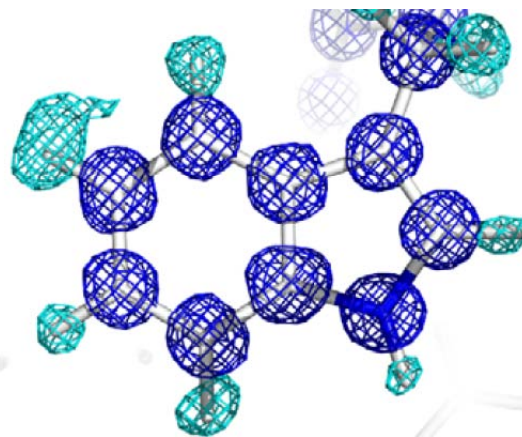


# HiPIP at 0.7 Å resolution by “low dose” data set: Electron densities

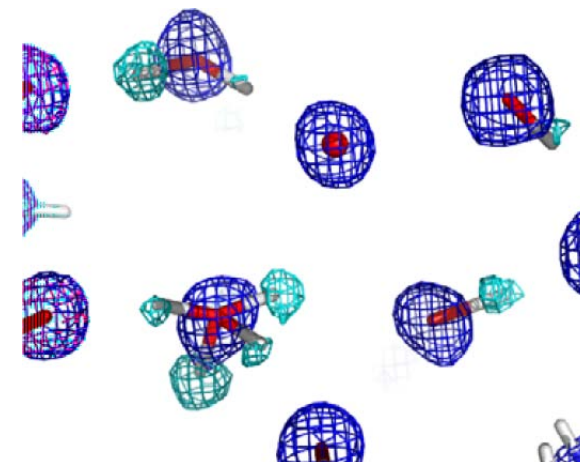
Arg28



Trp74

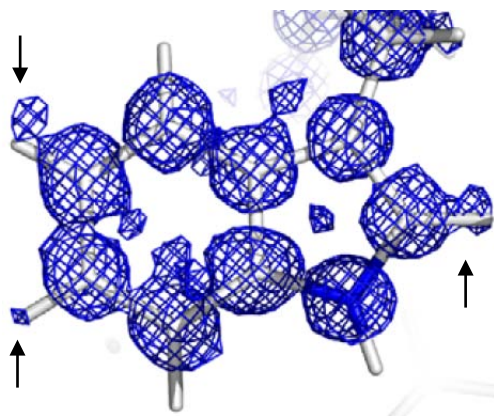


Waters



Blue: 2Fo-Fc contour 1.5  $\sigma$  (1.3 eÅ<sup>-3</sup>)

Light blue: Fo-Fc calculated by omitting hydrogen atoms  
contour 3.0 $\sigma$  (Arg28, Trp74), 2.5 $\sigma$  (Waters)



Trp74

Blue: 2Fo-Fc contour 0.8  $\sigma$  (0.7 eÅ<sup>-3</sup>)

# Charge density analysis in protein crystal structure

## Multipolar atom model

$$\rho_{\text{atom}}(r) = \rho_{\text{core}}(r) + P_{\text{val}} \kappa^3 \rho_{\text{val}}(\kappa r) + \sum \kappa'^3 R_{nl}(\kappa' r) \sum P_{lm} Y_{lm}$$

N.K. Hansen & P. Coppens, *Acta Cryst.* **A34**, 909-921 (1978).

$\rho_{\text{atom}}$ : the atomic electron density

原子の電子密度

$\rho_{\text{core}}$ : the spherical core electron density

内殻電子密度

$\rho_{\text{val}}$ : the spherical valence electron density

外殻電子密度

$P_{\text{val}}$ : the valence shell populations

外殻電子の存在確率

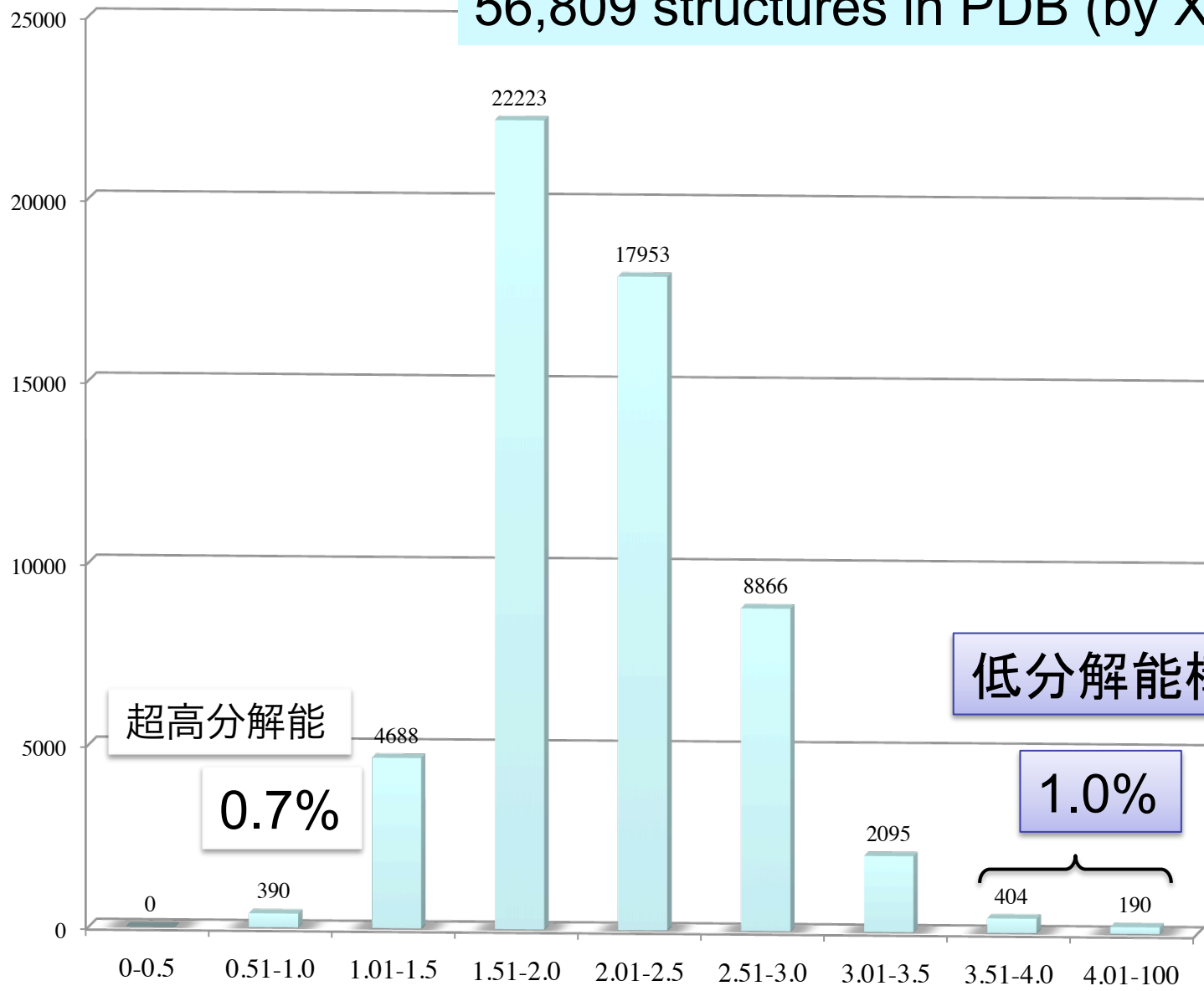
$P_{lm}$ : the multipole populations

多極子の存在確率

$\kappa, \kappa'$ : expansion-contraction parameter

電子密度の収縮を表す係数

# 56,809 structures in PDB (by X-ray)



超高分解能  
0.7%

低分解能構造解析

1.0%

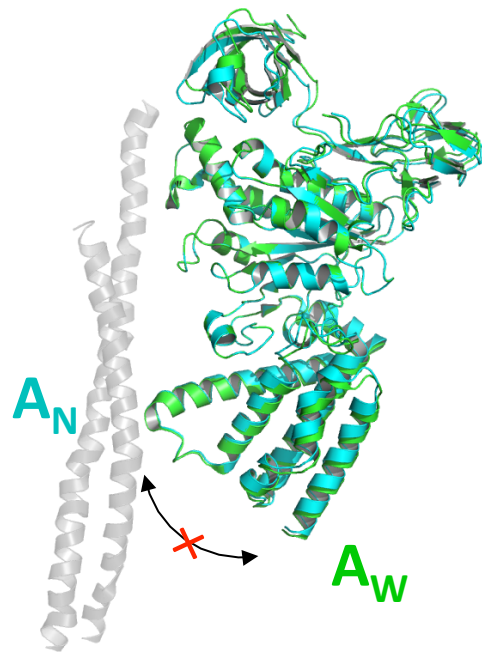
# 低分解能構造解析から得られる重要な構造情報

6 ~ 4 Å

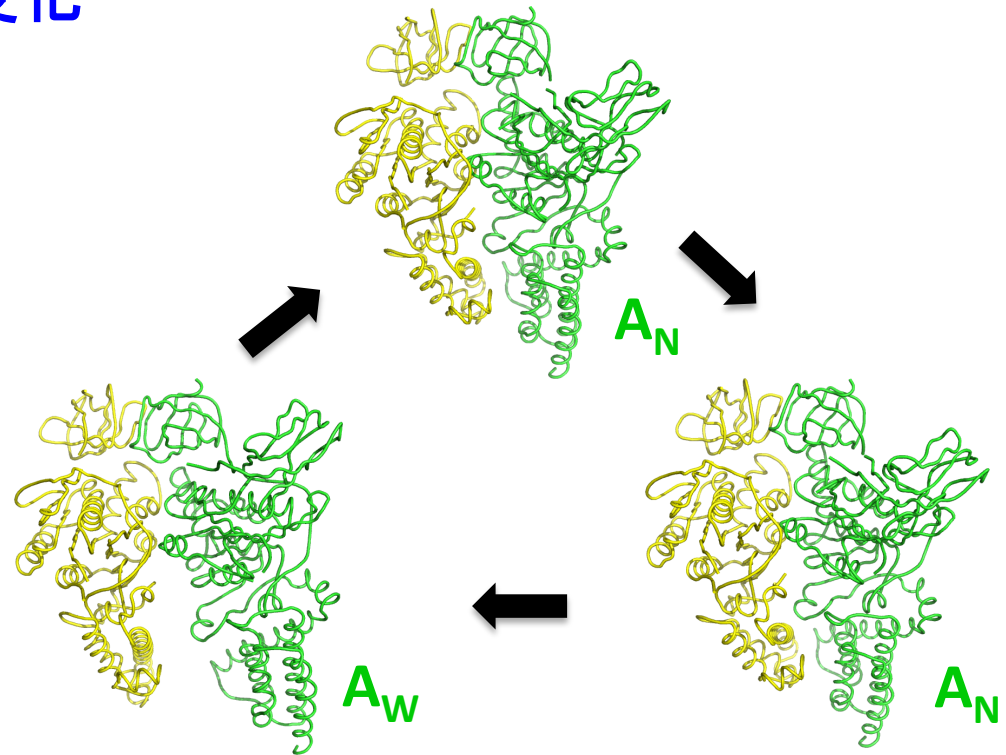
二次構造 ( $\alpha$ -helix)

低分子リガンド (基質, 補因子) の結合部位

三次, 四次構造変化



$V_1$ -ATPase, 4.5 Å



Numoto, N. *et al.*, *EMBO Rep.*, 2009.